

AMENDMENTS TO THE CLAIMS

Please cancel Claims 2, 4, 5, and 9. Please amend Claims 1, 6-8, 10, 11, 21, 22, and 24 as indicated below.

Claim 1 (currently amended): A method which is prognostic for a gastric preneoplastic/neoplastic disease afflicting a subject vertebrate, ~~wherein said disease affects a tissue, which tissue normally expresses MN/CA IX protein, but loses or has significantly reduced MN/CA IX expression upon carcinogenesis~~, said method comprising:

(a) detecting MN/CA IX protein or MN/CA IX polypeptide in a sample comprising gastric preneoplastic/neoplastic tissue sample comprising neoplastic cells taken from said vertebrate,

(b) quantitating the level of said MN/CA IX protein or MN/CA IX polypeptide in said sample,

(c) comparing the level of MN/CA IX protein or MN/CA IX polypeptide of step (b) to the average level of MN/CA IX protein or MN/CA IX polypeptide in comparable gastric preneoplastic/neoplastic tissue samples comprising neoplastic cells taken from vertebrates also afflicted by the same a gastric preneoplastic/neoplastic disease, wherein said vertebrates are of the same species as the subject vertebrate, and

(d) determining that said subject vertebrate has a ~~poorer~~ prognosis of shorter survival if the level of MN/CA IX protein or MN/CA IX polypeptide of step (b) is higher than the average level of MN/CA IX protein or MN/CA IX polypeptide in said comparable gastric preneoplastic/neoplastic tissue samples, than if said MN/CA IX protein or MN/CA IX polypeptide of step (b) were absent or at a significantly reduced level in said sample relative to said average level;

wherein said MN/CA IX protein is encoded by a nucleotide sequence selected from the group consisting of:

(1) SEQ ID NO: 1's coding region;

(2) nucleotide sequences that hybridize under stringent hybridization conditions of 50% formamide at 42 degree C. to complement of SEQ ID NO: 1's coding region; and

(3) nucleotide sequences that differ from SEQ ID NO: 1's coding region or from the nucleotide sequences of (2) in codon sequence due to the degeneracy of the genetic code;

wherein said MN/CA IX protein or said MN/CA IX polypeptide is specifically bound by the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128; and

~~wherein said tissue is selected from the group consisting of gastric mucosa, gallbladder, biliary ducts, ductal cells of duodenal glands, testis including ductular efferens and rete testis, ovary including surface coelomic epithelium and rete ovarii, basal cells of hair follicles, and central nervous system choroid plexus.~~

Claims 2-5 (canceled).

Claim 6 (currently amended): The method of claim 1 wherein said neoplastic disease is gastric cancer, and wherein said sample is taken from the invasion front of said gastric cancer.

Claim 7 (currently amended): The method of ~~claim 5~~ **claim 1** wherein said neoplastic disease is a gastric tumor, and said sample is taken from said gastric tumor and/or from a metastatic lesion derived from said gastric tumor.

Claim 8 (currently amended): The method of claim 1, wherein immunohistochemical staining with MN/CA IX-specific antibody is used to detect and quantitate MN/CA IX protein in the sample, and wherein the quantitating step (b) comprises determining an immunoreactivity score of cells in said sample comprising:

(b1) determining the percentage of immunoreactive cells, wherein the percentage of immunoreactive cells is assigned

a value of 0 if no immunoreactive cells,
a value of 1 if less than 10% immunoreactive cells,
a value of 2 if between 11% and 50% immunoreactive cells, or
a value of 3 if more than 50% immunoreactive cells;

(b2) determining the intensity of immunostaining of the immunoreactive cells, wherein the intensity of MN/CA IX immunostaining is assigned

- a value of 0 for staining equal to a negative control,
- a value of 1 for weak staining,
- a value of 2 for moderate staining, or
- a value of 3 for strong staining; and

(b3) adding the value for the percentage of immunoreactive cells found in step (b1) and the value for the intensity of immunostaining found in step (b2) to obtain the immunoreactivity score;

wherein the comparing step (c) comprises determining the immunoreactivity scores of said comparable samples analogously to the determination of the immunoreactivity score of the sample from the subject vertebrate in steps b(1) to b(3), and averaging said immunoreactivity scores from said comparable samples; and

wherein if the immunoreactivity score of the subject vertebrate sample determined in steps b(1) to b(3) is above the average immunoreactivity score of said comparable samples, concluding in step (d) that said vertebrate has a ~~poorer~~ prognosis **of shorter survival** than if said immunoreactivity score is at or below said average immunoreactivity score.

Claim 9 (canceled).

Claim 10 (currently amended): The method of claim 1, wherein said disease ~~is neoplastic and~~ comprises a **gastric** tumor, or a **gastric** tumor and one or more metastatic lesions derived from the **gastric** tumor, and wherein ~~a poorer~~ prognosis is measured in terms of shortened survival, ~~increased risk of recurrence of said neoplastic disease, or diminished or refractory response to treatment, following~~

treatment and/or surgical removal of the tumor, or the tumor and said one or more metastatic lesions.

Claim 11 (currently amended): The method of claim 1, wherein said **gastric** preneoplastic/neoplastic **tissue** sample is a formalin-fixed, paraffin-embedded tissue sample or a frozen tissue sample.

Claims 12 and 13 (canceled).

Claim 14 (previously presented): The method according to claim 1, wherein said detecting step (a) comprises the use of an assay selected from the group consisting of Western blots, enzyme-inked immunosorbent assays, radioimmunoassays, competition immunoassays, dual antibody sandwich assays, immunohistochemical staining assays, agglutination assays, fluorescent immunoassays cytofluorometry.

Claim 15 (canceled).

Claim 16 (original): The method according to claim 1, wherein said detecting step (a) comprises the use of the monoclonal antibody secreted by the hybridoma VU-M75 which has Accession No. ATCC HB 11128.

Claim 17 (canceled).

Claim 18 (previously presented): The method according to claim 1, wherein the detecting step (a) is by immunohistochemical staining, and wherein the quantitating step (b) comprises determining the percentage and/or the intensity of immunostaining of immunoreactive cells.

Claim 19 (original): The method of claim 1, wherein said vertebrate is a mammal.

Claim 20 (original): The method of claim 19, wherein said mammal is a human.

Claim 21 (currently amended): The method of claim 1, wherein said prognostic method is used as an aid in the selection of treatment for said gastric preneoplastic/neoplastic disease afflicting said vertebrate, further comprising concluding that said patient is a high risk patient in need of adjuvant therapies, if said determination in step (d) indicates a poorer prognosis of shortened survival.

Claim 22 (currently amended): The method of claim 1 wherein said sample is taken from the invasion front of said gastric preneoplastic/neoplastic disease, and said comparable samples are analogous invasion front samples.

Claim 23 (original): The method of claim 22 wherein said preneoplastic/neoplastic disease is a neoplastic disease.

Claim 24 (currently amended): A method which is prognostic for a gastric preneoplastic/neoplastic disease afflicting a subject vertebrate, wherein said disease affects is present in a gastric tissue in which 40% or more of the cells normally express MN/CA IX protein, but said gastric tissue loses MN/CA IX expression or expression of MN/CA IX is significantly reduced upon carcinogenesis, said method comprising:

- (a) taking a gastric tissue sample comprising neoplastic cells from the invasion front of said preneoplastic/neoplastic disease;
- (b) determining whether MN/CA IX protein or MN/CA IX polypeptide is absent or at a significantly reduced level in said gastric invasion front sample as compared to the level that said MN/CA IX protein or MN/CA IX polypeptide is normally expressed in said gastric tissue, when said gastric tissue is unaffected by said disease; and

(c) concluding that if said MN/CA IX protein or MN/CA IX polypeptide is neither absent nor at such a significantly reduced level in said invasion front sample, that the subject vertebrate has a ~~poorer~~ prognosis **of shorter survival** than if said MN/CA IX protein or MN/CA IX polypeptide were absent or at a such a significantly reduced level in said invasion front sample; and

~~wherein said tissue is selected from the group consisting of gastric mucosa, gallbladder, biliary ducts, ductal cells of duodenal glands, testis including ductular efferens and rete testis, ovary including surface coelomic epithelium and rete ovarii, basal cells of hair follicles, and central nervous system choroid plexus.~~